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TITLE: Demonstration That a mRNA Binding Protein is Responsible for
GADD45 mRNA Destabilization

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13. ABSTRACT (Maximum 200 Words) We are studying the post-transcriptional control of expression of the p53-inducible antiproliferative gene known as <u>G</u> rowth <u>A</u> rrest and <u>D</u> NA <u>D</u> amage induced gene 45 (GADD45). Using human breast carcinoma cell lines, we have demonstrated that the half-life of GADD45 mRNA is very responsive to ambient glutamine (GLN) availability. We have cloned the GADD45 cDNA and made several constructs of this cDNA that represent a first step toward making the constructs needed to test the region that is responsible for mRNA destabilization. We have also been developing the transfection techniques needed to conduct the mRNA stability studies. Unfortunately, the model system, TSE cells, has proven to be relatively difficult to transfect at a reasonable frequency. We are working to improve this frequency by using alternative transfection methods. Due to a shortage of technical help, the aims have not been completed in the first year. We have applied for a one-year no-cost extension to the award period.				
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Table of Contents

Cover.....1

SF 298.....2

Introduction.....4

Body.....4

Key Research Accomplishments.....4

Reportable Outcomes.....5

Conclusions.....5

References.....5

Appendices.....6

INTRODUCTION

We are examining the molecular mechanism by which the expression of an antiproliferative p53 downstream effector gene is post-transcriptionally controlled in breast cancer cells. The downstream effector gene to be studied is the growth arrest and DNA damage induced gene, GADD45. This gene is transcriptionally activated by wild-type p53; therefore GADD45 expression can be depressed in p53-deficient cells. Employing GADD45 knockout mice, A. J. Fornace and colleagues found that loss of GADD45 expression reproduced a large subset of the effects observed in p53 knockout mice (1). Our ultimate goal is to develop a technique that will upregulate expression of GADD45 in a p53-independent fashion. It is theorized that increasing GADD45 expression in p53-deficient cells will reproduce many of p53's antiproliferative functions. GADD45 expression in breast carcinoma cell lines is tightly controlled by the availability of the amino acid glutamine, primarily through a post-transcriptional mechanism (2). GADD45 mRNA is inherently unstable with a half-life of 30 to 45 minutes. Depriving these cell of media glutamine increased the half-life of GADD45 mRNA by approximately 17-fold. Conversely, repletion of media glutamine caused an immediate and rapid decay of GADD45 mRNA. Thus, this model system can be used to determine the mechanism by which GADD45 gene expression is controlled through mRNA turnover. In analogy to destabilization of AU-rich mRNAs such as c-myc by the AU-rich binding factor AUF-1 (3), it is hypothesized that there exists a mRNA binding protein that binds to GADD45 and causes or initiates its degradation.

BODY

There is very little progress to report at this time. This is due to a shortage of qualified laboratory personnel over this last year. The research technician who was originally to perform the laboratory procedures, Robyn Hassebrook, left the laboratory approximately one year ago. Since then I have conducted a national search for a postdoctoral fellow with no success. A part-time student employee has worked on the project, but progress so far has been very disappointing. For this reason I have requested a no cost extension for the award (see appendix).

We have cloned the GADD45 cDNA and made several constructs of this cDNA that represent a first step toward making the constructs needed to test the region that is responsible for mRNA destabilization. We have also been developing the transfection techniques needed to conduct the mRNA stability studies. Unfortunately, the model system, TSE cells, has proven to be relatively difficult to transfect at a reasonable frequency. We are working to improve this frequency by using alternative transfection methods.

KEY RESEARCH ACCOMPLISHMENTS:

- Constructed several GADD45 cDNA containing plasmid vectors.
- Evaluated several methods to transfect TSE cells.
- Optimized transfection by cationic lipid method.

REPORTABLE OUTCOMES:

- GADD45 cDNA plasmid constructs

REFERENCES

1. Hollander, M.C. et al. Disruption of gadd45 leads to genomic instability, loss of cellular growth control and radiation-induced carcinogenesis. *Proc Amer Assoc Cancer Res* 40:413 [abstract #2728], 1999.
2. S.F. Abcouwer, C. Schwarz and R. A. Mequid. Glutamine deprivation induces the expression of GADD45 and GADD153 primarily by mRNA stabilization. *J Biol Chem* 247:28645-28651, 1999.
3. Wilson, G.M. and G. Brewer. The search for trans-acting factors controlling messenger RNA decay. *Prog. Nucleic Acid Res Mol Biol* 62:257-91, 1999.

To: Shelley.marken@det.amedd.army.mil
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Date Sent: Thursday, May 16, 2002 8:17 AM

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Re: No-Cost Extension of Concept Award BC995864 (Award # DAMD17-01-1-0574).

Dear Ms. Marken,

I would like to request a no-cost extension to the duration of the Concept Award BC995864 (Award Number DAMD17-01-1-0574). The new end date would be 4/30/03.

The period of funding for this grant is, of course, one year and a progress report is due on May 31, 2002. Unfortunately, I have very little progress to report at this time. This is due to a shortage of qualified laboratory personnel over this last year. The research technician who was originally to perform the laboratory procedures, Robyn Hassebrook, left the laboratory approximately one year ago. Since then I have conducted a national search for a postdoctoral fellow with no success. A part-time student employee has worked on the project, but progress so far has been very disappointing.

As of this summer, I will have three pre-doctoral students working in the laboratory. I have also recently hired and trained an excellent research technician. Thus, I feel that I will be able to complete the project in the next year if allowed to do so. There is still ample money left in this account to complete the study as proposed during the next year.

Your help would be greatly appreciated.

Sincerely,

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